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Are land use and short time climate change effective on soil carbon compositions and their relationships with soil properties in alpine grassland ecosystems on Qinghai-Tibetan Plateau?

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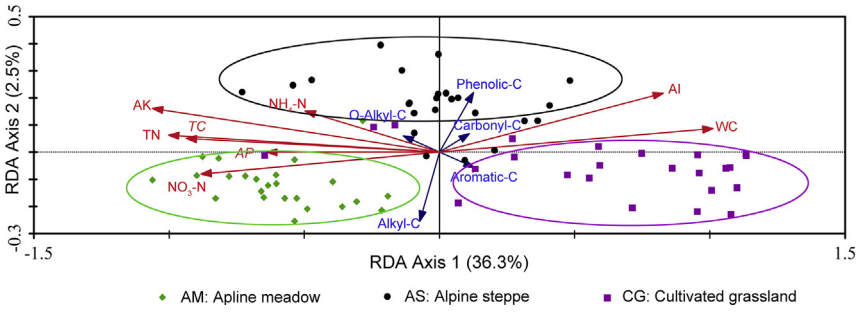
HIGHLIGHTS

- Grass plantation can promote the stability of soil organic carbon.
- Short-term climate change may not affect the molecular composition of the SOC.
- Land use can change the molecular composition of the SOC and soil nutrients.

GRAPHICAL ABSTRACT



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ABSTRACT

Fencing and grass plantation are two key interventions to preserve the degraded grassland on the Qinghai-Tibetan Plateau (QTP). Climate warming and N deposition have substantially affected the alpine grassland ecosystems. However, molecular composition of soil organic carbon (SOC), the indicator of degradation of SOC, and its responses to climate change are still largely unclear. In this study, we conducted the experiments in three types of land use on the QTP: alpine meadow (AM), alpine steppe (AS), and cultivated grassland (CG) under 2 °C climatic warming, 5 levels of nitrogen deposition rates at 8, 24, 40, 56, and 72 kg N ha^{-1} $year^{-1}$, as well as a combination of climatic warming and N deposition (8 kg N ha^{-1} $year^{-1}$). Our findings indicate that all three types of land use were dominated by O-alkyl carbon. The alkyl/O-alkyl ratio, aromaticity and hydrophobicity index of the CG were larger than those of the AM and AS, and this difference was generally stable under different treatments. Most of the SOC in the alpine grasslands was derived from fresh plants, and the carbon in the CG was more stable than that in the AM and AS. The compositions of all the alpine ecosystems were stable under short-term climatic changes, suggesting the short-term climate warming and nitrogen deposition likely did not affect the molecular composition of the SOC in the restored grasslands.

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1. Introduction

In recent decades, global changes including climate change, land use change and social-economic transformation have been driving the degradation of alpine grasslands, the dominant ecosystems on the Qinghai-Tibetan Plateau, which is well-known as the "Third Pole of the World" and the "Water Tower of the Asia" (Harris, 2010; Dong et al., 2010;

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Wang et al., 2015b). Fencing and grass plantation were two key interventions to preserve the degraded grassland with the implementation of Chinese government policies "Grassland Ban" (also termed as "Returning to Grassland by Excluding Grazing" or "Retire Livestock, Restore Pastures") (Dong et al., 2009, Dong et al., 2010). Up to now, many researchers have examined the plant biodiversity (Yang et al., 2014), soil physical and chemical properties (Dong et al., 2012; Liu et al., 2016a) or the carbon and nitrogen storage (Su et al., 2015; Zhang et al., 2016) to evaluate the effects of restoration practices in coping with land use changes and climate changes. However, few studies have been conducted to explore how the restoration practices under the changing environments impacted the components and stability of soil organic carbon (SOC) in the alpine grassland, which accounts for significant shares in China and global soil carbon, around 23.44% of China's total SOC and 2.5% of the global total soil carbon (Wang et al., 2002). It is widely reported that composition of SOC, the largest carbon pool on earth (Lal, 2004), varies greatly with the land uses across the world (Zhou et al., 2010; Pisani et al., 2015; Pisani et al., 2016; Wang and Zhong, 2016). Although the stabilization of SOC is mainly dominated by the elective preservation of recalcitrant organic components that accumulate in proportion to their chemical properties, it is still unpredictable as there are several mechanisms involved in SOC dynamics (Lützow et al., 2006). Some scholars believed chemical changes in organic materials would alter C stabilization in soil and reflect the stability of SOC (Angst et al., 2017).

Previous studies have revealed that vegetation, rather climate change, controls the SOM composition (Quideau et al., 2001; Feng et al., 2008). Organic detritus C, has a different chemical composition because of the different components of above-ground C, exhibits various decay speeds (Eviner and Chapin, 2003). Additionally, vegetation has significant effects on composition of soil microbe, which can decompose labile carbon to stable carbon. For example, Wang and Zhong (2016) found that soils dominated by coniferous species, which have more aliphatic compounds in their leaves, are higher proportion in alkyl C and a higher alkyl C/O-alkyl C ratio than the soils dominated by broadleaf species. Moreover, the aboveground plant community and soil properties have strong effects on soil microbial communities (Bardgett, 2005; Chu et al., 2016; Zhang et al., 2016), and thus may alter the SOM composition in the soil. In contrast, we assume that the climate change also impact the SOC composition and C stability. It was reported that global atmospheric temperature will rise by 1.5–4.8 °C by the end of this century (IPCC, 2013), nitrogen (N) deposition has continued to increase since last century (Liu et al., 2016b), both can influence the SOC content on a global scale (Feng et al., 2008; Pisani et al., 2015).

Climate change such as climate warming and N deposition might strongly affect the dynamics of carbon and nitrogen in soil, and the changes may lead to reduced CO_2 emission (Zhao et al., 2017). Long-term fertilization strategies have been shown to significantly influence the chemical composition of SOC (Li et al., 2015). Thus, future warming and N deposition could alter the composition of soil organic matter at the molecular level (Pisani et al., 2015). However, it is still largely unclear how warming and nitrogen deposition affect soil carbon stability (Feng et al., 2008; Feng et al., 2010; Pisani et al., 2014; Pisani et al., 2015), especially for the QTP's alpine grasslands in the era of rapid land use change. Thus, it is urgent to classify SOC molecular composition in the QTP's alpine grasslands and to study the molecular changes of SOC under warming and nitrogen deposition. Within this context, we conducted warming plus nutrient adding manipulative experiment to explore the pattern of SOC molecular composition in three types of grasslands, alpine meadow, alpine steppe, and cultivated grasslands on the QTP. We aimed to clarify the response of the QTP's alpine grasslands in the dimension of stability of soil carbon under land use change and climate change with two hypotheses: (1) the pattern of SOC molecular composition can be altered by land use; and (2) climate warming and nitrogen deposition can further change the SOC molecular composition.

2. Material and methods

2.1. Study sites

The study sites are located in Xihai Town, Haiyan County of Qinghai Province (36°56′N, 100°57′E, 3100 m ASL) and Tiebujia Town, Gonghe County of Qinghai Province (37°02′N, 99°35′E, 3270 m ASL) (Fig. 1). The alpine meadow (AM) is the major vegetation type dominated by *Kobresia capillifolia* in Haiyan County, and it is fenced since 2012. In Haiyan County, the mean annual precipitation is approximately 330–370 mm, and the mean annual temperature is approximately 1.4 °C. The alpine steppe (AS) dominated by *Stipa purpurea* and *Poa crymophila* is the major vegetation type of Gonghe County, and it is fenced since 2012. In Gonghe County, the mean annual precipitation is approximately 368.11 mm, and the mean annual temperature is approximately −0.7 °C. The cultivated grassland (CG) next to the alpine steppe in Tiebujia Town was planted with *Elymus nutans* in 2012.

2.2. Experimental design and sampling

2.2.1. Experimental design

Eight treatments were simulated: N1 deposition of ammonium nitrate (NH_4NO_3) at 8 kg N ha^{-1} $year^{-1}$ (corresponding to the annual N deposition on the QTP reported by Lu and Tian, 2007, N2 deposition of ammonium nitrate (NH_4NO_3) at 24 kg N ha^{-1} $year^{-1}$, N3 deposition of ammonium nitrate (NH_4NO_3) at 40 kg N ha^{-1} $year^{-1}$, N4 deposition of ammonium nitrate (NH_4NO_3) at 56 kg N ha^{-1} $year^{-1}$, N5 deposition of ammonium nitrate (NH_4NO_3) at 72 kg N ha^{-1} $year^{-1}$, warming treatment with open top chambers (OTC) at 2 °C warmer than the outside (W), and a combined treatment of warming with open top chambers (OTC) at 2 °C increment and N1 deposition (8 kg N ha^{-1} $year^{-1}$) (W&N1). In May of 2015, sets of 3 plots (replicates) in the size of 2 m × 5 m were randomly selected as replicates for each treatment in each type of grasslands, alpine meadow (AM) after 3 years fencing, alpine steppe (AS) after 3 years fencing and cultivated grassland (CG) after 3 years plantation. Meanwhile, three Open top chambers (OTCs) were randomly placed in the plots next to the nitrogen deposition plots for W treatment, another three OTCs (replicates) were randomly placed in the plots next to the nitrogen deposition plots for W&N1 treatment. Alpine meadow and alpine steppe are the main grassland types on the Qinghai-Tibetan Plateau (Fig. 1), and grassland fencing and grass plantation were two key interventions to preserve the degraded grassland (Dong et al., 2009). All the treatment areas were similar in their topographies and land use practices. The N was applied as ammonium nitrate (NH_4NO_3) in early May and early July of 2015.

2.2.2. Soil sampling and measurements

In the middle of August 2015, we collected 24 soil cores (8 treatments, 3 replicates for each treatment) at depths of 0–20 cm for each type of grassland. The samples were sealed in polyethylene bags and transported to the lab for extraction. Total nitrogen (TN) and total carbon (TC) were measured using an element analyzer (EA 3000, Italy). NH_4-N and NO_3-N were measured using a flow injection auto-analyzer (AACE, Germany). Five grams of soil was extracted with 50 ml of 0.01 M $CaCl_2$, and the solution was shaken for 1 h with a reciprocal shaker. It settled for 0.5 h after shaking, and the soil suspension was filtered using a filter paper. The concentrations of available potassium (AK) and available phosphorus (AP) were measured using inductively coupled plasma spectrometers (ICP; SPECTRO ARCOS EOP, Germany). Water content (WC) was measured using the wet soil and dry soil weights.

2.2.3. CPMAS-^{13}C NMR spectroscopy analysis

Soil samples were analyzed for SOC molecular composition by pretreating them with 10% (*v*/v) hydrofluoric acid (HF) and using NMR spectroscopy (Schmidt et al., 1997). Weigh 5 g soil sample in a

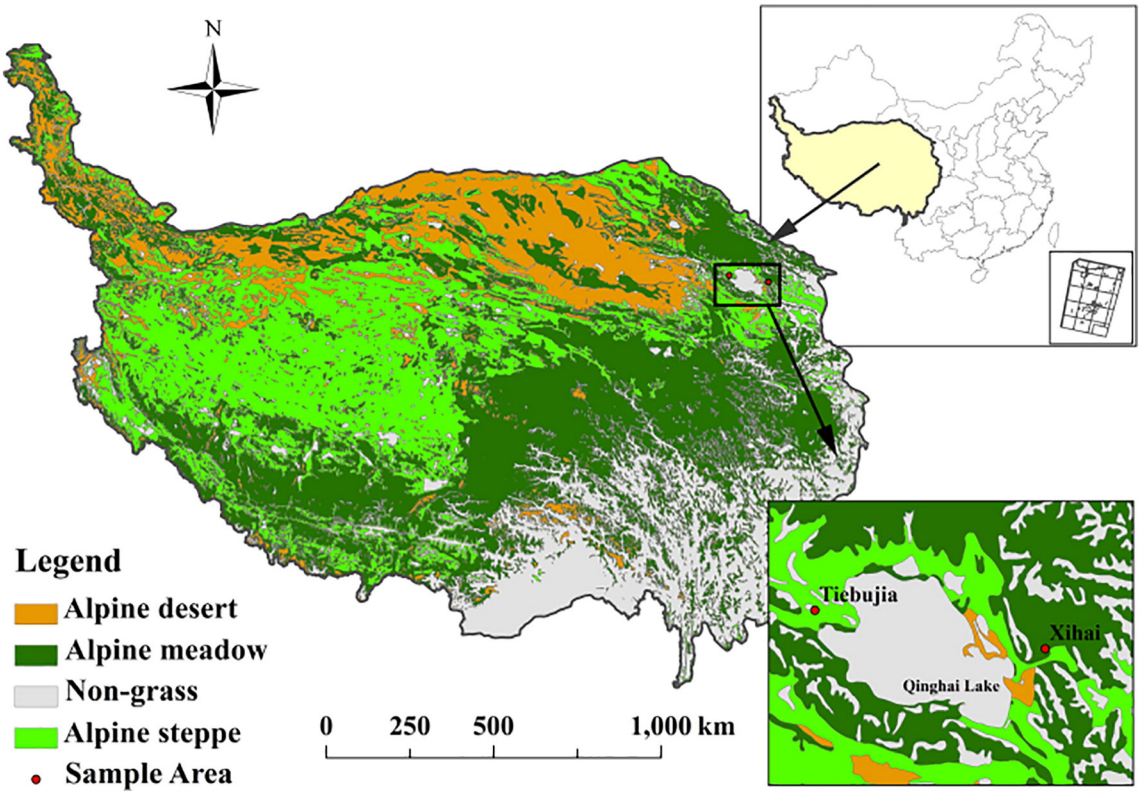

Fig. 1. Distribution of study sites on the Qinghai-Tibetan Plateau.

50 ml plastic centrifuge tube, add about 40 ml 10% (v/v) into the tube, then shaken for 12 h, repeated 4 times. Then washed with distilled water for 5 times to remove HF. Finally, the isolated sample was freeze-dried. The solid-state ^{13}C CPMAS NMR spectra of the soil samples were obtained at a frequency of 75.4 MHz on a Varian Infinity Plus 300 spectrometer. Chemagnetic 7.5 mm double resonance spectroscopy was used, and samples were spun at 9.0 kHz at the magic angle. A single contact time of 1.0 ms was used with an acquisition time of 4.0 s. Hexamethylbenzene was used to calibrate the chemical shifts.

The spectra were subdivided into five groups: (i) alkyl-C (0–50 ppm), (ii) O-alkyl-C (50–110 ppm), (iii) aromatic/double bond sp2-C (110–145 ppm), (iv) phenolic-C (145–165 ppm) and (v) carbonyl-C (165–190 ppm). The ratios of aromaticity and hydrophobicity were calculated as follows (Zhou et al., 2010):

Ratio of aromaticity = (aromatic-C + phenolic-C)/all resonances.

Ratio of hydrophobicity = (aromatic-C + phenolic-C + alkyl-C)/(O-alkyl-C and carbonyl-C).

The alkyl C was the most persistent fraction of SOM. Most alkyl C comes from original plant biopolymers (such as cutin, suberin and waxes) or from metabolic products of soil microorganisms (Ussiri and Johnson, 2003). As these materials decompose, the relative abundance of O-alkyl C in the litter materials decreases, and there is a progressive increase in alkyl C (Bonanomi et al., 2013). Therefore, the alkyl C/O-alkyl C ratio is an index of the decomposability of SOM (Wang et al., 2015a), and aromaticity and the hydrophobicity index indicate the degree of aromaticity and aliphatic properties, with larger ratios indicating a more aromatic and less aliphatic humic substance (Wang et al., 2010).

2.3. Statistical analysis

Two-way analysis of variance (ANOVA) was performed in SPSS 18 to identify significant differences in soil properties, SOC molecular composition, alkyl/O-alkyl ratios, aromaticity and hydrophobicity in response to different treatments and different land use. The relationship between soil properties and SOC molecular composition was examined using redundancy analysis (RDA) with the CANOCO 4.5 program (Microcomputer Power Company, Ithaca, New York, USA).

3. Results

3.1. Soil properties

All soil properties were strongly affected by land use (Table 1). The TC, TN, NH_4–N, NO_3–N, AK and AP were the lowest in the CG (Fig. 2b, d, f, h, j and l). The water content was highest in the CG, intermediate in the AS and the lowest in the AM (Fig. 2p). Climate change treatments had significant effects on soil TN, SOC, NO_3–N, AP and moisture (Table 1). The soil TC, TN, NH_4–N and AP under warming treatments were higher than others (Fig. 2a, c, e, and k), whereas that the NO_3–N (Fig. 2g) and water content (Fig. 2o) under warming treatments were lower than the others.

Table 1
Effects of land use (LU), experimental treatment (ET), and their interactions on soil properties.

	LU	ET	LU * ET
TC	24.71***	2.50*	1.73
TN	22.42***	3.71**	1.20
NH_4-N	31.77***	4.43***	1.62
NO_3-N	60.87***	5.61***	4.73
AP	9.27***	23.46***	1.08
AK	60.53***	0.57	3.35***
AI	142.64***	1.38	1.34
WC	63.68***	8.78***	3.56***

Note: The *F* value detected from a two-way ANOVA is listed.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

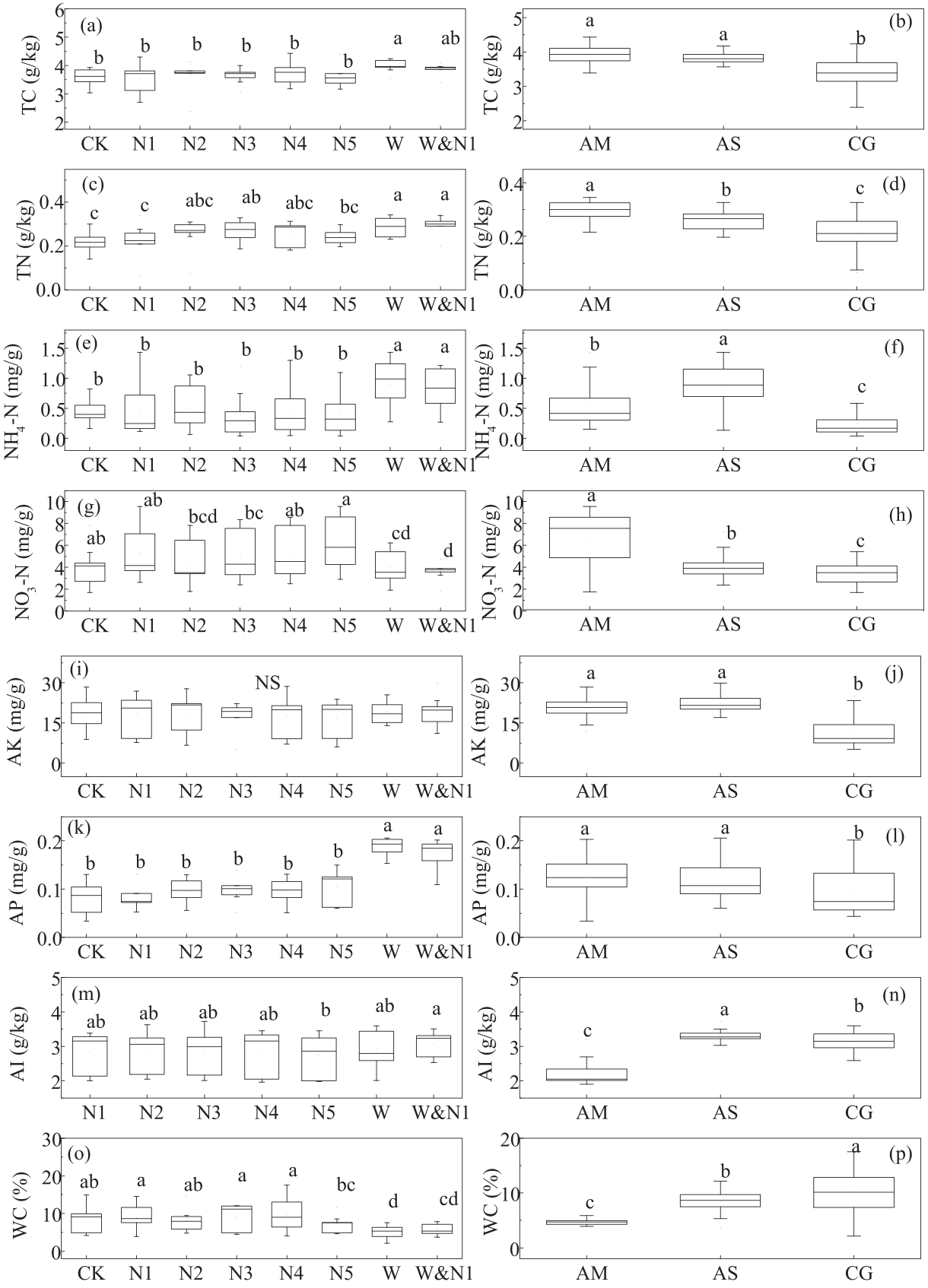

Fig. 2. Differences in soil properties among experimental treatments (no warming and no N deposition, CK; only N1 deposition, N1; only N2 deposition, N2; only N3 deposition, N3; only N4 deposition, N4; only N5 deposition, N5; only warming, W; warming and N deposition, W&N1) and land use (alpine meadow, AM; alpine steppe, AS; and cultivated grassland, CG). The boxes display the upper and lower quartiles of the data, and whiskers represent the standard deviation above and below the mean of the data. Different letters indicate significant differences at a $p < 0.05$ level across land use or experimental treatments.

Table 2
Effects of land use (LU), experimental treatment (ET) and their interactions on SOC molecular composition.*,**

	LU	ET	LU * ET
Alkyl-C	16.88***	1.17	1.38
O-Alkyl-C	15.84***	0.96	0.58
Aromatic-C	22.74***	0.62	1.04
Phenolic-C	19.23***	1.84	1.02
Carbonyl-C	13.36***	1.57	0.53
Aromaticity	31.27***	1.30	1.35
Hydrophobicity	9.14***	0.34	0.52

Note: The *F* value detected from a two-way ANOVA is listed.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

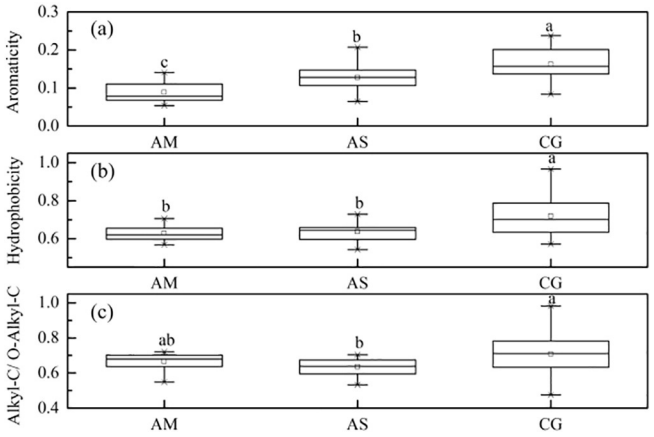

Fig. 4. Aromaticity, hydrophobicity and alkyl-C/O-alkyl-c of soil organic carbon (SOC) in three land use (alpine meadow, AM; alpine steppe, AS; and cultivated grassland, CG). The boxes display the upper and lower quartiles of the data, and whiskers represent the standard deviation above and below the mean of the data. Different letters indicate significant differences at a $p < 0.05$ level across land use.

3.2. Carbon molecular composition

The solid CPMAS-^{13}C NMR spectra of the different soils showed similar patterns, i.e., they were dominated by O-alkyl carbon and differed in the relative intensities of the different chemical shift regions (Fig. S1). Land use had strong effects on carbon molecular composition, whereas the treatments had no influence on carbon molecular composition (Table 2). The Alkyl C intensities were greater in the AM than the AS and CG (Fig. 3a), but the phenolic C intensities were lower in the AM than the AS and CG (Fig. 3d). The O-Alkyl-C intensities were lower in the CG than the AM and AS (Fig. 3b). The aromatic C and carbonyl-C intensities were highest in the CG, intermediate in the AS and the lowest in the AM (Fig. 3c and e).

Aromaticity and hydrophobicity were significantly higher in the CG than in the other land use (Fig. 4a and b). The CG had the highest alkyl/O-alkyl ratios, indicating that it was at a more advanced stage of degradation than the other soils (Fig. 4c).

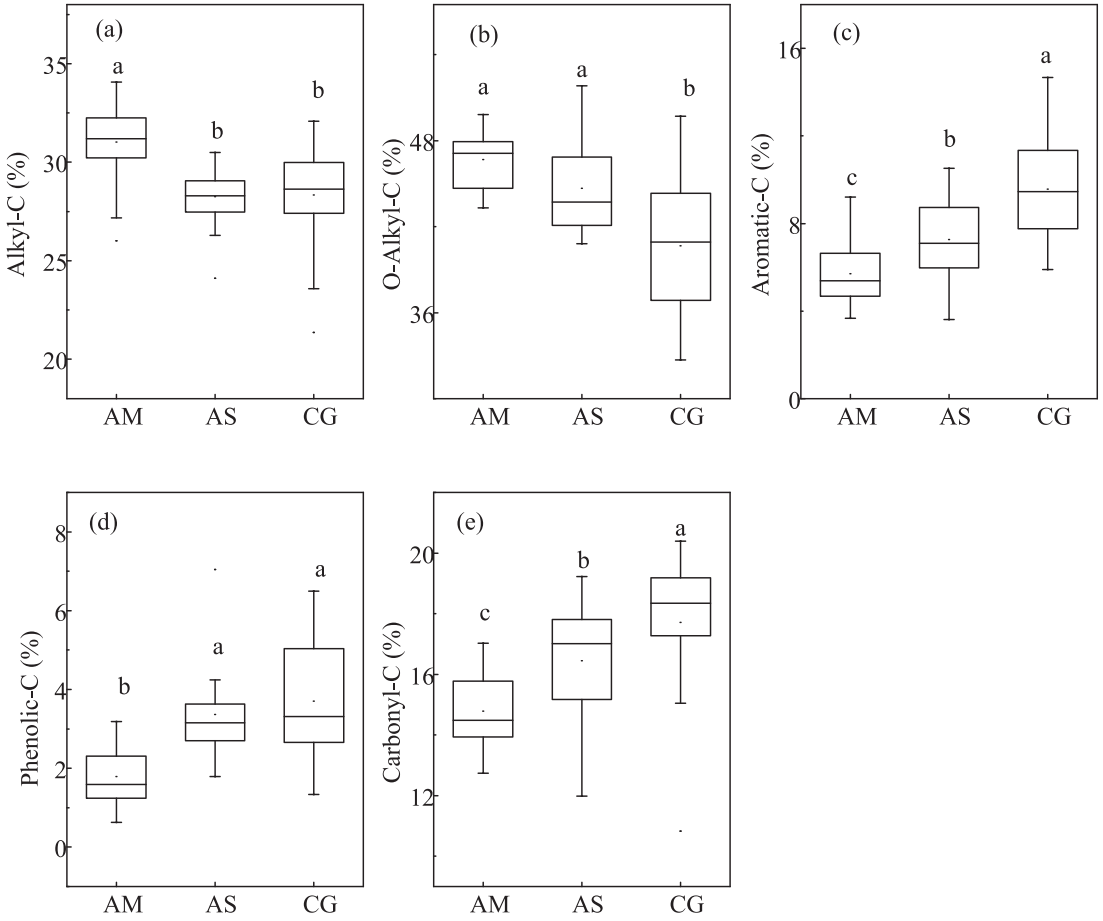

Fig. 3. Molecular compositions of soil organic carbon (SOC) in three land use (alpine meadow, AM; alpine steppe, AS; and cultivated grassland, CG). The boxes display the upper and lower quartiles of the data, and whiskers represent the standard deviation above and below the mean of the data. Different letters indicate significant differences at a $p < 0.05$ level across land use.

Table 3
The summary of RDA ordination and the species scores in RDA.

Parameters	Axis 1	Axis 2
Eigenvalues	36.31%	2.5%
Pseudo-F	5.19	
Significance	0.00	

3.3. Relationships between soil properties and soil carbon molecular composition

The RDA showed that significant predictors of soil properties account for 38.8% of the carbon molecular-level groups (Table 3). All the carbon molecular-level groups variations were explained by the first two axes (Table 3), with the first axis explaining >14-fold as much as the second axis (2.5%) in the RDAs (Fig. 5). TN, TC, AP, NH_4-N, AK and WC were the most important variables for the first axis, while NO_3-N and AI were the most important variables for the second axis (Table 4). Dynamics of the soil properties may be better related to the variations of O-alkyl C and aromatic C than those of other carbon molecular-level groups (Fig. 5). Soil nutrients had positive relationships with O-alkyl C and negative relationships with aromatic C. Soil water content was positively related to aromatic C. The nutrient content in the AM was higher than that in the AS and CG (Fig. 5). The O-alkyl C was highest in the AM, followed by the AS and CG, whereas the reverse order was observed for aromatic C in the dimension of the land use.

4. Discussion

We found that total soil nutrients (TN, TC and SOC) in the CG were significantly lower than in the AM and AS. In line with this result, the RDA results showed that the AM were higher in the nutrient content than the AS and CG. This is in agreement with the previous findings that the soil total nutrients in the alpine meadow were significantly higher than those in the alpine steppe (Zhang et al., 2016). We also found that the soil TC, TN, NH_4–N and AP under warming treatments were higher than those under nitrogen deposition treatment and the control treatment (Fig. 2). In the present study, the warming increased the soil carbon and nitrogen, with significant increase in both TC and TN (Fig. 2), implying warming could increase C sequestration by maintaining the stability of soil C stocks.

The RDA results in the present study further showed that soil nutrients had positive relationships with O-alkyl C, which were oxygen and nitrogen-substituted aliphatic constituents found in carbohydrates, peptides and methyl carbon found in lignin (Baldock et al., 1992; Simpson et al., 2008; Pisani et al., 2015). Aromatic C had negative and positive relationships with soil nutrients and O-alkyl C (Golchin et al., 1995). The present study can support previous researchers' (Zech et al., 1989) findings that soil water content is positively related to aromatic C. Our results showed that the alpine grasslands of the Qinghai-Tibetan Plateau are dominated by O-alkyl carbon, which is derived from fresh plant materials that decompose easily, e.g., carbohydrates and polysaccharides (Wang et al., 2015a). In the current study, we found that the peaks of the O-alkyl-C and aromatic signals were 104.91 and 130.94, respectively, corresponding to C-1 from cellulose, hemicellulose and lignin (Nierop et al., 1999). This implies that most of the SOC originated from fresh plant input (Li et al., 2015), and the microbial decomposition for SOC was low. These facts suggest that the input of fresh plant may greatly affect soil molecular composition of SOC.

The results of the present study confirmed our hypothesis that land use can change the patterns of SOC molecular composition. The CG had the highest aromaticity, hydrophobicity index and alkyl/O-alkyl ratio, in comparison to the AM and AS (Fig. 4), meaning that the vegetation has great effects on SOC chemical composition (Wang et al., 2010; Guo et al., 2016; Wang and Zhong, 2016). This can further support previous reports that the vegetation was the main factor controlling SOC molecular composition (Quideau et al., 2001; Wang et al., 2010; Wang and Zhong, 2016), due to the various chemical structures of the litter and the different soil microbial communities under different vegetation types (Quideau et al., 2001; Wang et al., 2010; Zhang et al., 2017). Previous studies revealed that tree species can significantly influence SOC chemical composition because of different aliphatic components (Wang and Zhong, 2016). Aliphatic components were assumed to be the main type of recalcitrant C in litter (Lützow et al., 2006), as they are less decomposable than other C components (Frouz et al., 2015), which vary greatly in alpine grassland plant. For example, the crude fat (EE) content in *Elynus nutans* was 1.72% (Zou et al., 2015), that in *Kobresia humilis* was 1.2% (Han et al., 1997), and those in *Poa crymophila* and *Stipa purpurea* were 1.07% and 1.9%, respectively. These are the dominant molecular-level groups in the CG, AM and AS, respectively. The differences in the aliphatic components of litter led to differences in the soil persist fractions, and thus slow the decomposition of SOC (Frouz et al., 2015). Moreover, the aboveground plant community and soil properties had strong effects on soil microbial communities, and over 70% of the variation could be explained by soil nutrients and moisture (Bardgett, 2005; Chu et al., 2016; Zhang et al., 2016). Fungi, which have mycelia networks and better mobility than soil bacteria (Bardgett, 2005), are considered to be the dominant decomposers of hard-to-decompose matter, such as cellulose and lignin. Thus, the abundance of fungi is the decisive factor controlling the decomposition of the lignin component of SOC (Wang et al., 2010; Angst et al., 2017). Zhang et al. (2016) revealed that the richness of soil fungi was highest in the CG, intermediate in the AS and the lowest in the AM, which may be the good reason to explain why the CG has relatively recalcitrant C components. Our findings suggest that the carbon in the grassland after grass plantation was more stable than that in fenced grasslands on the Qinghai-Tibetan Plateau.

Some researchers have revealed that the effects made by warming on soil organic carbon composition (Djukic et al., 2010; Wang et al., 2016; Pizzeghello et al., 2017) and warming changed the degradation

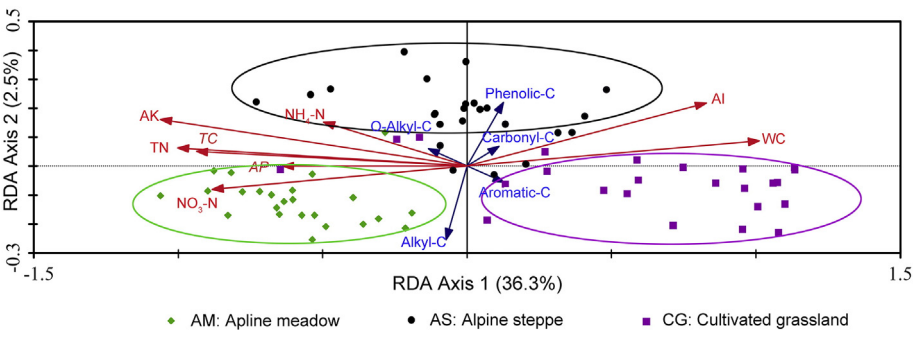

Fig. 5. Relationships between soil properties and carbon molecular-level groups according to the redundancy analysis (RDA).

Table 4
Correlations between environmental factors with the ordination axis.

Soil properties	Axis 1	Axis2	r^2	P
TN	−0.99	0.03	0.29	0.00
TC	−0.99	0.02	0.25	0.00
NH_4-N	−0.92	0.39	0.10	0.03
NO_3-N	−0.98	−0.19	0.24	0.00
AK	−0.98	0.17	0.34	0.01
AP	−0.99	−0.06	0.12	0.00
AI	0.91	0.39	0.30	0.00
WC	0.98	0.18	0.32	0.00

of soil organic materials through changing soil priming and the microbial community structure and activity (Pisani et al., 2015), such as positive response of fungi to warming (Treseder et al., 2016) or negative response to N fertilization (Frey et al., 2008), and alteration of the microbial communities in the mineral soil (Feng et al., 2010). However, we did not find significant difference in molecular of SOC relating to warming treatments and nitrogen deposition. That's to say, our findings from the present study did not support the second hypothesis that the pattern of SOC molecular composition can be altered by climatic warming and nitrogen deposition. The possible reason is that the aliphatic components of grass and the underground ecosystem of the alpine grasslands could be stable under short-term warming and nitrogen deposition treatments (Wang et al., 2014; Zhang et al., 2016). These findings, together with previous researchers' conclusions that the vegetation rather than climate change controls the SOM composition and C stability (Quideau et al., 2001; Feng et al., 2008) imply that the future short-term climate warming and nitrogen deposition will not increase the risk of carbon emission from the QTP's alpine grassland soils.

5. Conclusion

The cultivated grassland showed the lowest content of SOC and the highest C stability. In other words, grass plantation can promote the stability of soil organic carbon under climate changes after 3 years of restoration interventions. The grass plantation was better than the fencing in promoting the stability of SOC in the alpine region of the QTP. Key findings of this study are: 1) The carbon in the alpine grassland after grass plantation is more stable than that in fenced grasslands on the Qinghai-Tibetan Plateau. 2) Neither short time climate warming nor nitrogen deposition could change the molecular composition of the SOC in the restoration grasslands of QTP.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.12.206.

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